

## WEST Search History





DATE: Wednesday, February 25, 2004

Hide?	Set Name	Query	Hit Count
		<i>DB=USPT,DWPI; PLUR=YES; OP=ADJ</i>	
<input type="checkbox"/>	L1	6287778.pn. and (probe\$ same label\$)	2
<input type="checkbox"/>	L2	5945283.pn. and (dideoxynucleotide and label\$ and (polynucleotide or oligonucleotide))	2
		<i>DB=USPT,PGPB,EPAB,DWPI; PLUR=YES; OP=ADJ</i>	
<input type="checkbox"/>	L3	sorge-J\$.in. or arezi-B\$.in. or Hogrefe-H\$.in.	235
<input type="checkbox"/>	L4	L3 and composition	76
<input type="checkbox"/>	L5	L3 and (primer same probe)	46
<input type="checkbox"/>	L6	L5 and (interactive label or (biotin and streptavidin))	18
<input type="checkbox"/>	L7	primer same hybridiz\$ same target	7894
<input type="checkbox"/>	L8	primer same (first sequence and second sequence)	134
<input type="checkbox"/>	L9	L8 and probe	111
<input type="checkbox"/>	L10	L8 and (probe same primer same second sequence)	34
<input type="checkbox"/>	L11	(interactive label or (biotin same streptavidin) or (fluorescent resonance energy transfer or FRET))	17280
<input type="checkbox"/>	L12	(L9 and L11)	56
<input type="checkbox"/>	L13	probe near hybridiz\$ near primer	2042
<input type="checkbox"/>	L14	primer same ((first sequence and second sequence) or (first region and second region) or (first portion and second portion))	468
<input type="checkbox"/>	L15	L7 and L13	858
<input type="checkbox"/>	L16	L15 and (probe same (label or tag))	704
<input type="checkbox"/>	L17	L16 and (predetermin\$2 position)	17
<input type="checkbox"/>	L18	L16 and (polymorphism or SNP)	512
<input type="checkbox"/>	L19	chain terminator and L18	4
<input type="checkbox"/>	L20	L14 and (probe same hybridize same primer)	129
<input type="checkbox"/>	L21	L20 and L11	89
<input type="checkbox"/>	L22	primer extension	11046
<input type="checkbox"/>	L23	probe same (tag or FRET or interactive label or reporter molecule or (biotin and streptavidin))	8372
<input type="checkbox"/>	L24	L23 and L22	2036
<input type="checkbox"/>	L25	L24 and (chain terminator or ddATP or ddGTP or ddCTP or ddTTP)	184
<input type="checkbox"/>	L26	L25 and (primer same (first sequence or first region or first portion))	27

<input type="checkbox"/>	L27	5723591.pn. or 6277607.pn. or 6015675.pn. or 5578458.pn. or 5582989.pn.	9
<input type="checkbox"/>	L28	primer same uncomplementary same target	14
<input type="checkbox"/>	L29	L28 same probe	4
<input type="checkbox"/>	L30	L7 and L14	221
<input type="checkbox"/>	L31	L30 and L18	14
<input type="checkbox"/>	L32	L31 and probe	14
<input type="checkbox"/>	L33	L32 and L23	13
<input type="checkbox"/>	L34	single base extension	345
<input type="checkbox"/>	L35	L34 and ((FRET or fluorescence resonance energy transfer) and (mini-sequenc\$3 or minisequencing))	24
<input type="checkbox"/>	L36	L34 and (probe same (tag or reporter molecule or interactive label binding moiety or binding molecule))	105
<i>DB=PGPB,USPT,EPAB,DWPI; PLUR=YES; OP=ADJ</i>			
<input type="checkbox"/>	L37	tag and anti-tag or tag and antitag	2245
<input type="checkbox"/>	L38	L37 and (probe or primer or oligonucleotide)	2235
<input type="checkbox"/>	L39	L38 and (FRET or interactive label or reporter molecule or (biotin and streptavidin))	71
<input type="checkbox"/>	L40	L39 and (predetermin\$2 near (position or location))	25
<input type="checkbox"/>	L41	oligonucleotide same (immediately near (nucleotide or SNP or polymorphism or mutation or variant or mutant))	107
<input type="checkbox"/>	L42	L41 same ((antigen or biotin) and antibody)	0
<input type="checkbox"/>	L43	L41 same ((antigen or biotin) and \$avidin\$)	2
<input type="checkbox"/>	L44	oligonucleotide same (predetermin\$ near (location or position) and(nucleotide or SNP or polymorphism or mutation or variant or mutant))	177
<input type="checkbox"/>	L45	L44 and ((antigen or biotin) and \$avidin\$)	91

END OF SEARCH HISTORY

(FILE 'HOME' ENTERED AT 16:22:17 ON 25 FEB 2004)

FILE 'STNGUIDE' ENTERED AT 16:22:24 ON 25 FEB 2004

FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT' ENTERED  
AT 16:23:28 ON 25 FEB 2004

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L1      531 S SORGE J?/AU OR AREZI B?/AU OR HOGREFE H?/AU
L2      68 S L1 AND (PRIMER OR PROBE OR OLIGONUCLEOTIDE)
L3      14 S L2 AND (SNP OR POLYMORPH? OR MUTAT? OR VARIAN?)
L4      3 S L2 AND (INTERACTIVE LABEL# OR BIOTIN OR ?AVIDIN? OR TAG OR A
L5      3 S L2 AND (INTERACTIVE LABEL# OR BIOTIN OR STREPTAVIDIN? OR AVI
L6      456 S TAG AND ANTI-TAG
L7      83 S L6 AND (PROBE OR PRIMER OR SNP OR OLIGONUCLEOTIDE OR MUTANT
L8      0 S TARGET NUCLEIC ACID AND L7
L9      4 S L7 AND TARGET
L10     1 S L7 AND (CHAIN TERMINATOR OR DDATP OR DDGTP OR DDCTP OR DDTTP
L11     1 S L7 AND (PREDETERMINE? (5A) (LOCATION OR POSITION))
L12     32 DUP REM L7 (51 DUPLICATES REMOVED)
L13     0 S L12 AND TERMINAL END
L14     0 S L12 AND PRIMER EXTENSION
L15     2 S L12 AND KIT
L16     1264418 S (PROBE OR PRIMER OR SNP OR OLIGONUCLEOTIDE OR MUTANT)
L17     17510 S L16 AND (TAG OR BIOTIN OR INTERACTIVE LABLES OR FRET PAIR OR
L18     1568 S L17 AND LABEL
L19     100048 S L16 AND LABEL?
L20     1416 S L19 AND (TARGET (5A) (BIND? OR COMPLEMENT? OR ATTACH?))
L21     17 S L20 AND (TARGET (10A) NON-COMPLEMENT?)
L22     15 DUP REM L21 (2 DUPLICATES REMOVED)
L23     205 S DIDEOXYNUCLEOTIDE# (3A) LABEL?
L24     30 S L23 AND ((PROBE OR PRIMER OR POLYNUCLEOTIDE OR OLIGONUCLEOTI
L25     27 DUP REM L24 (3 DUPLICATES REMOVED)
L26     10 S L25 AND TARGET
L27     25 S L19 AND ((DIDEOXYNUCLEOTIDE OR DDATP OR DDCTP OR DDGTP OR DD
L28     16 DUP REM L27 (9 DUPLICATES REMOVED)
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Composition comprising nucleic acid molecules and a  
oligonucleotide capable of hybridizing with a portion  
of nucleic acid, and comprises a modified nucleotide at or  
near the 3'-terminal nucleotide;  
DNA detection and quantification by polymerase chain  
reaction using DNA primer

AUTHOR: NAZARENKO I; RASHTCHIAN A; SOLUS J; PIRES R M; DARFLER M;  
GEBEYEHU G; ASTATKE M  
PATENT ASSIGNEE: INVITROGEN CORP  
PATENT INFO: WO 2002057479 25 Jul 2002  
APPLICATION INFO: WO 2001-US50460 27 Dec 2001  
PRIORITY INFO: US 2001-330468 23 Oct 2001; US 2000-748146 27 Dec 2000  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
OTHER SOURCE: WPI: 2002-627370 [67]  
AN 2003-01971 BIOTECHDS

61528266  
6258266  
607/69/58

L26 ANSWER 5 OF 10 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN  
ACCESSION NUMBER: 2003-02845 BIOTECHDS  
TITLE: Analysis of methylation of cytosine bases in genomic DNA  
samples isolated from human sources, by utilizing bisulfite  
treatment and fluorescence polarization assay techniques;  
DNA primer, DNA probe, DNA chip and bioinformatics for  
high throughput screening and disease diagnosis  
AUTHOR: BERLIN K; DISTLER J  
PATENT ASSIGNEE: EPIGENOMICS AG  
PATENT INFO: WO 2002061124 8 Aug 2002  
APPLICATION INFO: WO 2002-EP923 29 Jan 2002  
PRIORITY INFO: DE 2001-1004938 29 Jan 2001; DE 2001-1004938 29 Jan 2001  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
OTHER SOURCE: WPI: 2002-674825 [72]  
AN 2003-02845 BIOTECHDS  
AB DERWENT ABSTRACT:

Determining identity of a polymorphic nucleotide (N1) in a  
    **target** nucleic acid, by using a **probe**  
    having a **labeled** nucleotide which specifically base  
    pairs with N1 and which is covalently attached to the  
    amplicon using an enzyme;  
        human 5-hydroxytryptamine receptor-type-2a SNP detection  
        using polymerase chain reaction

AUTHOR: JONES K; LEUTHER K K; SHAPERO M H  
PATENT ASSIGNEE: SMITHKLINE BEECHAM CORP  
PATENT INFO: EP 1256632 13 Nov 2002  
APPLICATION INFO: EP 2002-76698 2 May 2002  
PRIORITY INFO: US 2001-289606 7 May 2001; US 2001-289606 7 May 2001  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
OTHER SOURCE: WPI: 2003-021220 [02]

AN 2003-04741 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - Determining identity of a polymorphic nucleotide (N1) in a  
**target** nucleic acid (T1), by amplifying T1 with bound  
locus-specific primer pairs, contacting the amplification product (P1)  
with a **labeled probe** comprising a detecting  
nucleotide (DN) which specifically base pairs with N1, in the presence of  
an enzyme that catalyzes covalent bond formation between DN and P1, and  
detecting the label.

DETAILED DESCRIPTION - A method (M1) of determining the identity of  
a polymorphic nucleotide, comprising: (a) contacting under hybridizing  
conditions, a **target** nucleic acid comprising a polymorphic  
site, and a solid substrate comprising one or more bound locus-specific  
primer pairs; (b) amplifying the **target** nucleic acid with the  
locus-specific primer pair, where the amplifying results in an  
amplification product bound to the solid support at each end; (c)  
contacting the amplification product with a **labeled**  
**probe** comprising at least one detecting nucleotide that will  
specifically base pair with the polymorphic nucleotide, in the presence  
of an enzyme that catalyzes the formation of a covalent bond between the  
detecting nucleotide and the amplification product; and (d) detecting the  
label, where the identity of the label on the detecting nucleotide  
indicates the complement of the polymorphic nucleotide.

BIOTECHNOLOGY - Preferred Method: In M1, the enzyme is DNA  
polymerase. The amplification product is denatured and contacted with an  
extension primer that hybridizes to a site immediately adjacent to the  
polymorphic nucleotide, prior to contacting with the **labeled**  
**probe**, where DNA polymerase extends from the extension primer to  
covalently attach a **labeled probe** to its 3' end. The  
amplification product is cleaved with an endonuclease to generate a free  
end, and cleaving with a distance-cleaving endonuclease, results in a  
cleavage product having an overhang strand and a recessed strand  
comprising a 3' terminus, where the polymorphic nucleotide is on the  
single-stranded overhang of the cleavage product. The recessed strand  
provides an extension primer for the DNA polymerase. The amplification  
product is contacted with labeled probes selected from the group  
consisting of at least two differentially **labeled**  
**dideoxynucleotides**. Mutually distinguishable extension primers  
are used. The enzyme is ligase. Alternatively, the amplification product  
is cleaved with an endonuclease to generate a free end, and cleaving the  
amplification product with a distance-cleaving endonuclease, results in a  
cleavage product having a single-stranded overhang strand and a recessed  
strand, where the recessed strand has a 3' terminus, and the polymorphic  
nucleotide is on the single-stranded overhang of the cleavage product.  
The recessed strand is contacted with ligase and at least one nucleotide  
complementary to the polymorphic nucleotide under conditions that permit  
covalent linkage. The amplification product is contacted with  
differentially **labeled oligonucleotide** probes  
selected from the group consisting of all possible sequences of the

single-stranded over-hang. At least two different labels are used. The polymorphic nucleotide is the first nucleotide on the single-stranded overhang of the cleavage product. The amplification product comprising the detecting nucleotide is released from the substrate for detection. The amplification product comprising the detecting nucleotide is detected in situ. The solid substrate comprises a capture primer.

USE - The method is useful for determining the identity of a polymorphic nucleotide in a complex mixture of nucleic acids where one or more distinct polymorphisms can be present in the mixture, and multiple polymorphisms can be screened in parallel.

ADVANTAGE - Each locus to be tested does not have to undergo an initial separate amplification or a solution-based multiplexed amplification. Numerous polymorphic sites can be analyzed simultaneously in a single reaction chamber.

EXAMPLE - The genotyping approach required a Type IIS restriction enzyme site to be precisely positioned within one of the polymerase chain reaction (PCR) primers. In order to determine the accuracy of various Type IIS enzymes, artificial DNA duplexes were prepared on beads and used as enzyme substrates. The specification shows the sequence of a duplex containing recognition sites for BbvI and BsmFI. These digested duplexes were used as templates in minisequencing reactions with individual FAM-ddNTPs. Reaction products were released by EcoRV digestion and resolved on 20 % 1 x TBE acrylamide gels. The fluorescent gel image showed only the expected nucleotide FAM-ddUTP was incorporated into the duplex. Sybr Green I staining of the gel showed equal amounts of digested duplex was released from all the beads. Thus BbvI accurately cleaves an artificial duplex immobilized to the surface of acrylamide microspheres. To assess the specificity of the genotyping scheme, a 135 bp amplicon spanning the T102C polymorphism in the human gene for the 5-hydroxytryptamine type 2a (5-HT2a) receptor (Warren, et al. (1993) Hum. Mol. Genet. 2:338) located on chromosome 13q was evaluated using a single color single nucleotide polymorphism (SNP) minisequencing format. There are several general features of the primer design for solid-phase amplification and genotyping. Each primer-pair designed for a specific polymorphic locus introduced a StuI site and a PvuII site into the resulting PCR product. In addition, the 6 base pair (bp) Type IS BbvI restriction enzyme site, embedded within locus-specific sequence, was always positioned 12 nucleotides away from the SNP, either in the forward primer or in the reverse primer. The site was placed in the forward primer for the 5-HT2a model amplicon, resulting in ddNTP incorporation into the non-coding strand. The BbvI site was used only in conjunction with the StuI site. StuI digestion linearized the bound PCR product generating blunt ends that could not serve as templates for ddNTP incorporation. BbvI digestion of the linearized product released a short segment containing the BbvI recognition sequence, as well as exposed the polymorphic nucleotide in a 5' overhang on a fragment attached to the bead. Extension of the 3'-hydroxyl group of the recessed nucleotide with a single fluorescent ddNTP led to incorporation at the position of genetic variation. Fluorescently labeled fragments were released from the beads by cleavage with PvuII. Individual beads containing a 5-HT2a primer-pair were used for **target** hybridization and solid-phase amplification with genomic DNA template from a single individual. Restriction enzyme digests with MspI and BpmI in addition to direct sequencing of 5-HT2a PCR products confirmed the genotype as a C/T heterozygote. Following StuI and BbvI cleavage, beads were used in minisequencing reactions with individual FAM-ddNTPs. Fluorescent signal was observed only from the FAM-ddATP and FAM-ddGTP labeling reactions, corresponding to incorporation into the opposite strand of the T and C polymorphisms. Sybr Green I staining of the gel after fluorescent imaging showed that equal amounts of 5-HT2a-PCR product were synthesized and released from each bead. The results show that BbvI can accurately cleave a linearized solid-phase amplification product, which can then serve as a template for ddNTP incorporation. (28 pages)

Analyzing variant site of **target** nucleic acid,  
involves hybridizing primer to **target** nucleic acid,  
conducting template-dependent extension of primer, detecting  
presence or absence of double-labeled extension product;  
DNA primer for mutation and SNP detection

AUTHOR: HUNG S; GLAZER A N; MATHIES R A  
PATENT ASSIGNEE: DNA SCI INC  
PATENT INFO: US 6573047 3 Jun 2003  
APPLICATION INFO: US 2000-547292 11 Apr 2000  
PRIORITY INFO: US 2000-547292 11 Apr 2000; US 1999-129129 13 Apr 1999  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
OTHER SOURCE: WPI: 2003-874210 [81]  
AN 2004-03200 BIOTECHDS  
AB DERWENT ABSTRACT:

NOVELTY - Analyzing a variant site of a **target** nucleic acid,  
involves hybridizing primer bearing fluorophore to the **target**  
nucleic acid to form a labeled hybrid, conducting template-dependent  
extension of primer in the presence of a polymerase and non-extendible  
nucleotide, and detecting presence or absence of the double-labeled  
extension product indicating the identity of the nucleotide at the  
variant site.

DETAILED DESCRIPTION - Analyzing (M1) a variant site of a  
**target** nucleic acid, involves: (a) hybridizing a primer bearing a  
first fluorophore to a segment of the **target** nucleic acid to  
form a labeled hybrid, where the 3'-end of the primer hybridizes to the  
**target** nucleic acid immediately adjacent to the variant site; (b)  
conducting several of template-dependent extension reactions with  
different primers, where different primers hybridize adjacent different  
variant sites on **target** nucleic acids; or (c) hybridizing a  
primer bearing a first fluorophore to a segment of the **target**  
nucleic acid to form a labeled hybrid, where the 3'-end of the primer  
hybridizes to the **target** nucleic acid adjacent to the variant  
site, conducting template-dependent extension of the primer in the  
presence of a polymerase and at least one dideoxynucleotide (ddNTP)  
bearing a second fluorophore. INDEPENDENT CLAIMS are also included for:  
(1) determining (M2) the identity of a nucleotide at a variant site of a  
**target** nucleic acid, involves hybridizing a primer bearing a  
first fluorophore to a segment of the **target** nucleic acid to  
form a labeled hybrid, where the 3'-end of the primer hybridizes to the  
**target** nucleic acid immediately adjacent to the variant site,  
conducting template-dependent extension of the primer in the presence of  
a polymerase and at least one non-extendible nucleotide bearing a second  
fluorophore, where a double-labeled extension product is formed if the  
non-extendible nucleotide is complementary to the nucleotide at the  
variant site and the first and second fluorophore borne by the extension  
product are brought into an energy transfer relationship while primer is  
hybridized to the **target** nucleic acid, where the first and  
second fluorophore comprise a donor and an acceptor fluorophore which  
have a donor-acceptor spacing in the extension product of less than 18  
nucleotides, the donor fluorophore having a high extinction coefficient  
and a low fluorescence quantum yield, and detecting the presence or  
absence of the double-labeled extension product, the presence or absence  
of double-labeled extension product indicating the identity of the  
nucleotide at the variant site; and (2) determining (M3) the identity of  
a nucleotide at a site within a **target** nucleic acid, involves  
hybridizing a primer containing a first fluorophore to a segment of the  
**target** nucleic acid to form a labeled hybrid, where the 3'-end of  
the primer hybridizes to the **target** nucleic acid adjacent to  
the site, conducting template dependent extension of the primer with a  
polymerase by mixing a labeled non-extendible nucleotide linked to a  
second fluorophore and optionally one or more extendible nucleotides  
complementary to the nucleotide(s) of the **target** nucleic acid  
located between the primer 3' end and the variant site with the labeled



hybrid under conditions appropriate for primer extension, where an energy transfer (ET) labeled nucleic acid product is formed while the primer is hybridized to the **target** nucleic acid if the non-extendible nucleotide is complementary to the nucleotide at the site, and where the first and second fluorophore comprise a donor and an acceptor fluorophore which have a donor-acceptor spacing in the ET-labeled product of less than 18 nucleotides, and detecting the presence or absence of ET-labeled product, the presence or absence of ET-labeled product indicating the identity of the nucleotide at the variant site.

WIDER DISCLOSURE - A kit for performing (M1) are also disclosed.

BIOTECHNOLOGY - Preferred Method: In (M1), detecting comprises detecting double-labeled extension product while the primer remains hybridized to the **target** nucleic acid. The detecting comprises optically exciting the donor fluorophore and detecting an increase or decrease in fluorescence emission by the acceptor or donor fluorophore due to resonance energy transfer between the donor and acceptor fluorophore. (M1) further comprises separating the double-labeled extension product from other components in the extension reaction before detecting double-labeled extension product. Separating comprises performing a size based separation. The size based separation is chosen from HPLC and electrophoresis. The primer bears an attachment moiety and separating comprises allowing the primer to attach to a support through the attachment moiety. The donor fluorophore is chosen from 5 and 6-carboxyrhodamine-110 (R110), 6-carboxyrhodamine-6G (R6G), N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA), 6-carboxy-X-rhodamine (ROX), 6-carboxyfluorescein (FAM), 6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein (JOE), 5-carboxy-2',4',5',7'-tetrachlorofluorescein (ZOE), 6-carboxy-2',4,7,7'-tetrachlorofluorescein (TET), 6-carboxy-2',4,4',5',7,7'-hexachlorofluorescein (HEX), NAN, Texas Red and Rhodamine Red. The donor fluorophore is cyanine. The donor or acceptor fluorophore is attached to the primer and the acceptor or donor fluorophore is attached to the non-extendible nucleotide. The first fluorophore is attached to an internal nucleotide, modified nucleotide or a nucleotide substitute. The nucleotide substitute or modified nucleotide is chosen from universal linker or modified thymidine. The donor-acceptor spacing is 3-10 nucleotides or 4-6 nucleotides. The non-extendible nucleotide is chosen from arabinoside triphosphate and a dideoxynucleotide. Preferably, the non-extendible nucleotide is a **dideoxynucleotide**. The secondary **label** is attached to the 5'-end of the primer and is chosen from a mass label, a radioisotope, a chromophore, a magnetic particle, an electron dense agent, and a metal chelate. Preferably, the secondary label is a mass label. Different primers bear different mass labels. The mass labels comprise one or more monomers and the different mass labels are composed of a different number of the monomers. The different variant sites are different sites on the same **target** nucleic acid or different sites on different **target** nucleic acids and the extension reactions are conducted in a single reaction vessel. (M1) further comprises separating the different extension products before detecting the presence or absence of different extension products, separation being accomplished by HPLC or electrophoresis. The different variant sites are located on different **target** nucleic acids and each extension reaction is conducted in a separate reaction vessel, and further comprises collecting extension product from the reaction vessels before detection. (M1) further comprises separating the different extension products before detecting the presence or absence of different extension products, separation being accomplished by HPLC or electrophoresis. In (M1), the conducting step is performed with a single dideoxynucleotide. The conducting step comprises mixing the labeled hybrid with at least two ddNTPs, each type of ddNTP bearing different labels. The conducting step comprises mixing the labeled hybrid with ddATP, ddGTP, ddCTP and ddTTP. The secondary label is a mass label such that different primers bear different mass labels and the method further comprises separating the different extension products according to size. In (M1), each variant site is a biallelic site, each

extension reaction is conducted with two labeled non-extendible nucleotides that are complementary to the two nucleotides potentially at the variant site and bear different second fluorophores, and the mass label and the second fluorophore borne by the extension product indicates the identity of the nucleotide at the variant site. The different extension products are separated on a single lane of an electrophoretic gel. In (M1), each extension reaction is conducted with labeled non-extendible nucleotide analogs of dATP, dTTP, dCTP and dGTP that bear different second fluorophores, and the mass label and the second fluorophore borne by the extension product indicates the identity of the nucleotide at the variant site. In (M2), the cyanine dye is chosen from 3-(epsilon-carboxypentyl)-3'-ethyl-5,5'-dimethyloxacarbocyanine (CYA), Cy2, Cy3, Cy3.5, Cy5, Cy5.5, Cy7, and Cy7.5. The acceptor dye has a high quantum yield and a large extinction coefficient. In (M3), the ET-labeled product is separated from the other components in the extension reaction followed by detection. The separation is an electrophoretic separation. The first fluorophore is a donor or acceptor molecule and the second fluorophore is an acceptor or donor molecule.

USE - The method is useful for a variety of application such as analyzing point mutations and single nucleotide polymorphisms (SNPs). The method is useful for other applications in which specific sequence information is of value, including detection of pathogens, paternity disputes, prenatal testing and forensic analysis. The method is useful for developing correlations between certain genotypes and patient prognosis. The method is useful for formulating optimal treatment protocols for a particular disease. The method is useful for assessing the actual risk of an individual known to be susceptible of acquiring a disease. The method is useful for identifying point mutations in microorganisms that could potentially result in altered pathogenicity or resistance to certain therapeutics. The method is useful for identifying carriers of mutant alleles, tissue classification or in blood typing.

ADVANTAGE - The method permits multiple analyses to be conducted simultaneously and at high throughput. (24 pages)

L26 ANSWER 3 OF 10 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN

ACCESSION NUMBER: 2003-24341 BIOTECHDS

TITLE: Detecting **target** sequences by hybridizing probe pairs having elements with distinct electrophoretic mobility/elution characteristics to a **target**, ligating the end subunits of the elements, and isolating by electrophoresis/chromatography;  
DNA probe hybridization for multiple selected sequence detection

AUTHOR: GROSSMAN P D; FUNG S; MENCHEN S M; WOO S L; WINN-DEEN E S

PATENT ASSIGNEE: PE CORP NY

PATENT INFO: US 2003073108 17 Apr 2003

APPLICATION INFO: US 2002-167337 10 Jun 2002

PRIORITY INFO: US 2002-167337 10 Jun 2002; US 1992-862642 3 Apr 1992

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2003-677984 [64]

AN 2003-24341 BIOTECHDS

AB DERWENT ABSTRACT:

NOVELTY - Detecting sequences in **target** polynucleotide (I), involves adding to (I), probe pairs (PP) containing two probe elements (PEs) complementary to selected sequences in (I), where one PE contains a polymer chain that imparts distinctive electrophoretic mobility or elution characteristic, hybridizing PP with (I), ligating end subunits of PEs, and releasing and separating PP by electrophoresis or chromatography.

DETAILED DESCRIPTION - Detecting (M1) the presence or absence of a number of selected **target** sequences in a **target** polynucleotide, involves: (a) adding to a **target** polynucleotide (I), a number of different sequence probe pairs (PP), where each PP

includes two polynucleotide probe elements which are complementary in sequence to adjacent portions of a selected one of the **target** sequences in (I), one of the elements in a probe pair contains a nonpolynucleotide polymer chain which imparts a distinctive electrophoretic mobility in a sieving matrix, to the associated probe pair, when the elements in the pair are ligated, and the other, second element in the pair contains a detectable reporter **label**, hybridizing the **probe** pairs with (I), treating the hybridized polynucleotides under conditions effective to ligate the end subunits of **target**-bound probe elements when their end subunits are base-paired with adjacent **target** bases, releasing the ligated probe pairs from (I), and separating the released, ligated probe pairs by electrophoresis in such a sieving matrix; or (b) adding PPs to (I), where one of the elements in PP contains a nonpolynucleotide polymer chain which imparts a distinctive elution characteristic in a chromatographic separation medium, to the associated probe pair, when the elements in the pair are ligated, and the other, second element in the pair contains a detectable reporter label, carrying out hybridization and ligation reactions as above, releasing the ligated probe pairs from (I), and separating the released, ligated probe pairs by chromatography in such a chromatographic medium. INDEPENDENT CLAIMS are also included for: (1) distinguishing (M2) different-sequence polynucleotides electrophoretically in a sieving medium, by forming one or more different-sequence polynucleotide(s), each different-sequence polynucleotide containing a detectable reporter label and an attached polymer chain which imparts to each different-sequence polynucleotide, a distinctive electrophoretic mobility in a sieving matrix, fractionating the polynucleotide(s) by capillary electrophoresis in a sieving matrix, and detecting the fractionated polynucleotide(s); and (2) a probe composition (PC) for use in detecting one or more of a number of different sequences in a **target** polynucleotide, comprises a number of sequence-specific probes, each characterized by a binding polymer having a probe-specific sequence of subunits designated for base-specific binding of the polymer to one of the **target** sequences, under selected binding conditions, and attached to the binding polymer, a polymer chain which imparts to each probe, a distinctive electrophoretic mobility in a sieving matrix.

Amplifying single nucleotide polymorphisms (**SNP**),  
useful as markers for the identification of genomic regions  
associated with complex diseases in humans comprises  
generating at least one nick translate molecule that  
comprises an **SNP**;

**SNP** detection by DNA amplification, nick  
translation and DNA microarray analysis useful for disease  
marker identification

AUTHOR: MAKAROV V L; LANGMORE J P  
PATENT ASSIGNEE: RUBICON GENOMICS INC  
PATENT INFO: WO 2003002752 9 Jan 2003  
APPLICATION INFO: WO 2002-US20200 25 Jun 2002  
PRIORITY INFO: US 2001-302172 29 Jun 2001; US 2001-302172 29 Jun 2001  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
OTHER SOURCE: WPI: 2003-210281 [20]  
AN 2003-10614 BIOTECHDS  
AB DERWENT ABSTRACT:

NOVELTY - Amplifying a single nucleotide polymorphism (**SNP**)  
from a DNA sample comprises generating at least one nick translate  
molecule, which comprises **SNP**, from a DNA sample.

DETAILED DESCRIPTION - Amplifying a **SNP** comprises: (a)  
obtaining the DNA sample comprising the **SNP** to be amplified;  
(b) generating at least one nick translate molecule, which comprises  
**SNP**, from a DNA sample; and (c) amplifying the nick translate  
molecule. INDEPENDENT CLAIMS are included for the following: (1) a method  
for producing library of **SNP**-containing DNA molecules; (2) a  
method for analyzing a **SNP** from DNA samples; (3) a method for  
isolating a specific **SNP**-containing nick translate molecule;  
(4) a method for isolating a complementary nucleic acid molecule to a  
specific **SNP**-containing nick translate molecule; (5) a method  
for amplifying a nucleic acid sequence for **SNP** analysis; (6)  
methods for multiplex amplification of nucleic acid sequences for  
**SNP** analysis; (7) methods for multiplex amplification of nucleic  
acid sequences comprising **SNP** of interest; (8) methods for  
assaying a DNA sample for the presence of multiple specific SNPs; (9)  
methods for analyzing at least one **SNP** from individuals, or  
from DNA samples from individuals; and (10) a method for amplification of  
genome comprising **SNP** of interest.

BIOTECHNOLOGY - Preferred Method: In the amplification method,  
generating the nick translate molecule comprises attaching upstream  
adaptor molecules to ends of DNA sample molecules to provide a nick  
translation initiation site, subjecting the DNA molecules to nick  
translation comprising DNA polymerization and 5'-3' exonuclease activity  
to produce the nick translate molecules, and attaching downstream adaptor  
molecules to the nick translate molecules to produce adaptor attached  
nick translate molecules. Producing library of **SNP**-containing  
DNA molecules or analyzing a **SNP** from DNA samples comprises  
obtaining a DNA sample comprising at least one **SNP**, digesting  
DNA molecules of the DNA sample with a sequence-specific endonuclease,  
attaching upstream adaptor molecules to ends of DNA molecules of the  
sample to provide a nick translation initiation site, subjecting the DNA  
molecules to nick translation comprising DNA polymerization and 5'-3'  
exonuclease activity to produce the nick translate molecules that  
comprise the **SNP**, attaching downstream adaptor molecules to the  
nick translate molecules to produce adaptor attached nick translate  
molecules, and separating the **SNP**-containing nick translate  
molecules. The separation is by size or hybridization. This separating  
step further comprises amplification of at least one of the **SNP**  
-containing nick translate molecules. The amplification is by polymerase  
chain reaction. For the analysis, the upstream adaptors are  
non-identical. Isolating a specific **SNP**-containing nick  
translate molecule comprises: (a) obtaining **SNP**-containing nick  
translate molecules; (b) ligating to an end of the **SNP**

-containing nick translate molecules a first **oligonucleotide** to form a first **oligonucleotide**-nick translate molecule complex, where the first **oligonucleotide** comprises nucleic acid sequence complementary to an adaptor end of the nick translate molecules, a double stranded region that facilitates the formation of an adjacent hairpin or loop in the **oligonucleotide**, a free 3' OH, and a 5' phosphate;

(c) attaching to the complex a second **oligonucleotide** to form of a first **oligonucleotide**-nick translate molecule-second **oligonucleotide** complex, where the second **oligonucleotide** comprises nucleic acid sequence adjacent to an adaptor end of the nick translate molecules, nucleic acid sequence non-identical to a restriction endonuclease site used in generating the nick translate molecules, and an affinity tag; and (d) isolating the nick translate molecule-first **oligonucleotide**-second **oligonucleotide** complex from the nick translate molecules for by the affinity tag. The attaching step further comprises ligation of the second **oligonucleotide** to the first **oligonucleotide**-nick translate molecule complex. The first **oligonucleotide** further comprises a labile base. The double-stranded region of the first **oligonucleotide** is approximately at least about 6-8, preferably 4 bases, or no more than 100 bases. The nucleic acid sequence of the second **oligonucleotide** that corresponds to the nucleic acid sequence of adjacent to an adaptor end of the nick translate molecule is five nucleotides in length. The affinity tag of the second **oligonucleotide** is biotin. Isolating a complementary nucleic acid molecule to a specific **SNP**-containing nick translate molecule comprises: (a) obtaining nick translate molecules; (b) introducing to the molecules an **oligonucleotide** comprising a nucleic acid sequence complementary to a specific region of the specific nick translate molecule, a nucleic acid sequence substantially non-identical to a sequence in the specific nick translate molecule, and an affinity tag, where the **oligonucleotide** hybridizes to the specific nick translate molecule; (c) extending the **oligonucleotide** by polymerization to form a complementary nucleic acid molecule for the specific nick translate molecule; and (d) isolating the extended complementary nucleic acid sequence molecule for the nick translate molecules. This method further comprises amplifying the complementary nucleic acid molecule, where the amplification is by polymerase chain reaction. The **oligonucleotide** further comprises a hairpin or loop structure.

Amplifying a nucleic acid sequence for **SNP** analysis comprises: (a) generating a nick translate molecule comprising the nucleic acid sequence, and an upstream and downstream adaptor; and (b) performing polymerase chain reaction to amplify the nick translate molecule using a first **oligonucleotide** complementary to an adaptor sequence of the nick translate molecule and a second **oligonucleotide** complementary to a known nucleic acid sequence of the nick translate molecule. The multiplex amplification of nucleic acid sequences for **SNP** analysis comprises: (a) generating nick translate molecules comprising nucleic acid sequence comprising the **SNP**, where each nick translate molecule comprises a first and second adaptor; (b) introducing to the nick translate molecules the first **oligonucleotide** complementary to the first or second adaptor sequence, and the second **oligonucleotide** that is complementary to the known nucleic acid sequence of a nick translate molecule; and (c) amplifying the region in the nucleic acid sequence of the nick translate molecules between the first and second **oligonucleotide** by polymerase chain reaction. The second **oligonucleotide** can further comprise a nucleic acid sequence complementary to the second adaptor, and multiple nucleotide bases at the 3' terminal end of the second **oligonucleotide**, which are complementary to corresponding multiple nucleotide bases in the nucleic acid sequence of the nick translate molecule immediately adjacent to the second adaptor. The multiple nucleotide bases comprise two or three bases. The multiplex amplification of nucleic acid sequences comprising **SNP** of

interest comprises: (a) obtaining a DNA sample; (b) processing the DNA sample to generate a library of nick translate molecules, where the nick translate molecules are separated into sub-libraries of molecules that are complementary to specified positions within the region of the DNA and the sub-libraries are partitioned into chambers of a solid support, or where the nick translate molecules are in a pooled collection and comprise of sequences complementary to unknown positions within a region of the template DNA; and (c) amplifying by polymerase chain reaction within the chambers or pooled collection, at least one nick translate molecule or its fragment using a **primer** from the known nucleic acid sequence. The DNA sample further comprises a genome. The solid support is a microwell plate. The pooled collection is in a single tube. This method further comprises applying the amplified nick translate molecules to a DNA microarray, where the hybridization of a nick translate molecule to the DNA microarray identifies the **SNP**.

Assaying a DNA sample for the presence of multiple specific SNPs comprises: (a) generating nick translate molecules from the DNA molecules of the sample, where the nick translate molecules comprise the multiple SNPs; (b) introducing to the nick translate molecules the oligonucleotides that hybridize adjacent to a specific **SNP** location, and where the 3' base of the **oligonucleotide** is variable; (c) extending by polymerization from the **oligonucleotide**, where extension only occurs if the variable 3' base of the **oligonucleotide** is complementary to the corresponding nucleotide of the specific **SNP**; and detecting the extended **oligonucleotide**. The detection further comprises separation by size, is preferably by capillary electrophoresis. The extended **oligonucleotide** is detected by detecting a **label** on the 3' base of the **oligonucleotide**. The **label** is preferably fluorescent. The multiple specific SNPs are detected concomitantly. The **labels** for multiple non-identical oligonucleotides are distinguishable. Alternatively, this method comprises: (a) generating nick translate molecules comprising the **SNP** from the DNA molecules of the sample; (b) introducing to the nick translate molecules the first **oligonucleotide** that hybridizes such that its 5' end is adjacent to a specific **SNP**; (c) extending the first **oligonucleotide** by **primer** extension to form nick translate molecule-first **oligonucleotide** extension product hybrids; (d) introducing to the hybrids the second **oligonucleotide** that hybridizes adjacent to the specific **SNP** and comprises a variable 3' end; (e) ligating the 3' end of the second **oligonucleotide** to the 5' end of the first **oligonucleotide** extension product, where the ligation occurs only if the variable nucleotide is complementary to the **SNP**, to form, a ligated molecule of the first and second **oligonucleotide** extension products; and (f) detecting the ligated molecule. The second oligonucleotides are fluorescently or differentially fluorescently **labeled**. Analyzing at least one **SNP** from individuals comprises generating at least one specific nick translate molecule comprising the **SNP** from DNA samples from each individual, and detecting the **SNP**. The detection comprises the step of the assay cited above. Analyzing at least one **SNP** from DNA samples from individuals comprises: (a) generating from each of the DNA samples a specific nick translate molecule comprising **SNP**, where an adaptor on one end of the nick translate molecule comprises a unique nucleic acid sequence; (b) introducing to the nick translate molecules a two-part **oligonucleotide** comprising a first part having nucleic acid sequence complementary to the unique nucleic acid sequence of the adaptor and a second part having nucleic acid sequence complementary to the nucleic acid sequence immediately 5' to the **SNP**, where the introduction results in the hybridization of the two parts of the **oligonucleotide** to the respective complementary sequences of the nick translate molecule and results in the formation of a loop in the nick translate molecule to bring the two parts in proximity with each

other; (c) introducing the two-part **oligonucleotide** the differentially **fluorescently labeled dideoxynucleotide** triphosphates and DNA polymerase, or which is complementary to the **SNP**; and (d) detecting the **SNP**. The detection further comprises hybridization of the **fluorescently labeled dideoxynucleotide** triphosphatase-incorporated two-part **oligonucleotide** to a solid support that comprises multiple positions with unique adaptor sequence. The solid support is preferably a chip. The amplification of a genome comprising **SNP** of interest comprises obtaining the genome, generating the nick translate molecules from the genome, and amplifying the **SNP**-containing nick translate molecule. This method further comprises detecting the **SNP** by microarray analysis, sequencing, hybridization, or their combination.

USE - The methods are useful for detecting single nucleotide polymorphisms, which are particularly useful as markers for the identification of genomic regions associated with complex diseases in humans.